Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

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Product screening test for Kasumin and Phyton

Part 1: Growth rate test.

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A report prepared for

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PLANT & FOOD RESEARCH *Pseudomonas syringae* pv. *actinidiae* (Psa) RESEARCH NOTE

KEY PROJECT DETAILS

Project Title	*In vitro* product testing
Project Leader	Kerry Everett
Research Requested / Contracted by	Etec
Date (Month, Year)	December 2011
Based on information as at	December 2011

RESEARCH QUESTION AND AIM

Treatments to limit the spread of Psa in orchards are required. Etec have requested preliminary *in vitro* testing of three products for activity against *Pseudomonas syringae* pv. *actinidiae*. This document reports on the growth rate testing of two of those products.

Aim: To carry out in vitro tests on products for potential efficacy for control of New Zealand isolates of *P. s.* pv. *actinidiae*

METHODOLOGY (Include brief details of experimental design, methodology and protocols)

Experimental design

P. s. pv. *actinidiae* was isolated from leaf and flower tissue collected from KPIN 7668 (RP2, KEP3) that has been haplotyped as Psa-V. Single cells from the isolation plates were used to generate the test cultures. A test measuring the growth of the bacteria in the presence of the test products was conducted. Details of the products evaluated are shown in Table 1.

Methods and protocols

Growth test

Products to be tested were adjusted to twice the following concentrations: 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml. A 2-ml aliquot of each of these concentrations of product was added to 15-ml tubes containing 2 ml of 2x concentration nutrient broth, to result in concentrations of 1x nutrient broth and 1x 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml products. A suspension of culture KEP3 that had been grown on King's medium B agar (KB) (King et al. 1954) for 24 hours at 25ºC were spread onto fresh Petri plates containing the same medium. After 44 hours at 25ºC, bacterial cells were harvested by washing with sterile deionised water (SDW) and the concentration spectrophotometrically determined. The concentration was adjusted to 10^3 cfu/ml and a 100-µl aliquot was added

to the tubes containing products and nutrient broth. There was an uninoculated control for all product concentrations, and there was an inoculated control in 1x nutrient broth. After 44 hours of growth at 25°C, 100 µl was removed from each tube (except for the biological products) and placed in a cuvette containing 2.4 ml of deionised water. The optical density was measured at 535 nm. The efficacy against Psa was calculated by logit transforming the OD 535 values, where logit = ln {(p/1-p)} and p = the proportion of the OD 535 values in unamended nutrient broth. Any negative values were adjusted to zero. Logit values were plotted against the logarithmic transformation of product concentration. The response was thus linearised. The slope of the linear portion of the transformed data was calculated by linear regression. The effective concentration at which growth was inhibited by 50% (EC_{50} value) was calculated from each linear regression equation for Y = 0, and when Y = - 2.77 for EC_{95} values. A constant value was added to non-transformed data to enable 0 and 100% values to be used in the calculations.

A low EC_{50} value indicates an effective product. If the EC_{95} value is also low, then the EC_{50}/EC_{95} ratio will be close to 1. This indicates total kill at a low dosage and this is a good product.

Table 1: Products tested

Product name	active ingredient	% or concentration
Kasumin 2L	Kasugamycin hydrochloride	23 g/L
Phyton® 27AG	Copper sulphate pentahydrate	253 g/L

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

Growth test

Psa growth was almost completely inhibited at high concentrations (100 and 1000 µg/ml) of both tested products (Fig. 1).
The EC50 and EC95 values are displayed in Table 1.

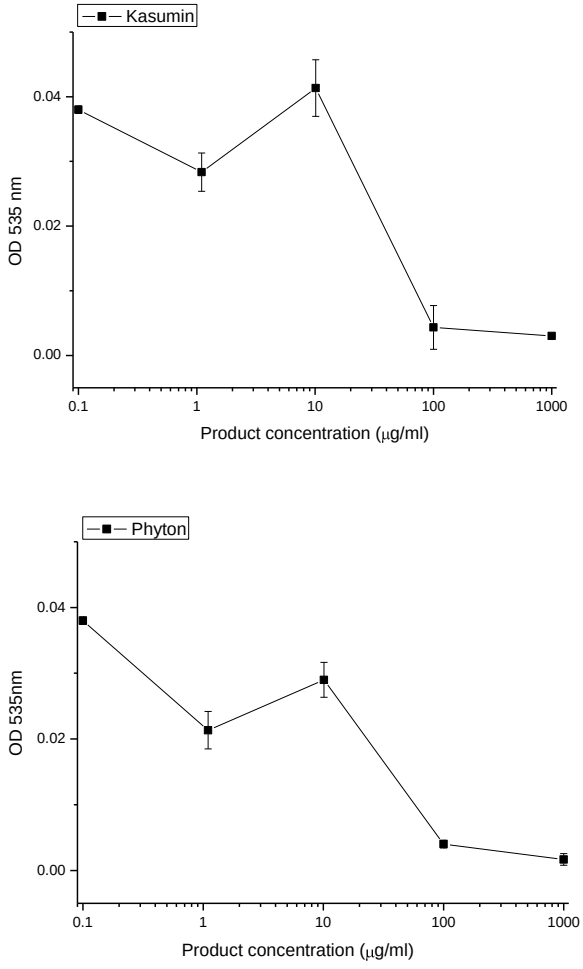

Figure 1. Optical density at 535 nm after 44 hours of Psa growing in the presence of chemical products at 25°C, plotted against concentration. The value of 0.1 is zero plus a constant value so that this value can be included.

Table 2. Effective concentration (EC) in µg/ml at which bacterial cells are reduced by 50% (EC_{50}), 95% (EC_{95}) and a ratio of EC_{50}/EC_{95} as determined by absorbance at 535 nm after 44 hours of growth at 25°C.

	Effective Concentration (EC) in µg/ml		
Product name	EC_{50}	EC_{95}	EC_{50}/EC_{95}
Kasumin	22.1	169.9	0.13
Phyton	22.6	156.1	0.14

key to abbreviations – see Table 1.

RECOMMENDATIONS FOR INDUSTRY

In order to validate the results of these tests, field testing is required, as environmental conditions and the presence of plant material can affect the efficacy of these products.

CONCLUSIONS

Both products tested were effective against Psa in this 'in vitro' growth test.

FUTURE RESEARCH STEPS

Small inoculated kiwifruit plants could be used to test these materials further in the laboratory or the glasshouse. Field spray trials should also be conducted to test these promising products further.

REFERENCES

King EO, Ward MK, Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescin Journal of Laboratory and Clinical Medicine 44: 301-307.

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This report has been approved by:

Kerry Everett
Scientist
Date: December 2011